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Metachromatic Leukodystrophy in the Habbanite Jews: High Frequency in a Genetic Isolate and Screening for Heterozygotes

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SUMMARY

A very high incidence of late infantile metachromatic leukodystrophy (MLD) (1/75 live births) was found in the Jewish Habbanite community which constitutes a genetic isolate of about 1,000–1,200 individuals. Screening in this population for aryl sulfatase A (ASA) levels in married adults revealed a carrier frequency for MLD of 17% and identified six couples of whom both partners were heterozygotes (6% of screened couples). In three pregnancies of these couples, prenatal diagnosis for the detection of ASA in the fetus was performed.

INTRODUCTION

Metachromatic leukodystrophy (MLD) is an autosomal recessive disorder [1] associated with the failure to degrade cerebroside sulfate (sulfatide) as a result of the deficiency of the lysosomal hydrolase ASA [2], which is necessary for the hydrolysis of the sulfated esters of the sulfatide [3]. As a consequence, sulfatide accumulates in the brain and peripheral nerves as well as in some visceral organs [4–6]. MLD is a genetically heterogeneous group of disorders classified into three distinct types, namely: the late infantile, which is the most common, the juvenile, and the adult. The first symptoms in the late infantile type appear between 12 and 18 months: the child's gait becomes unsteady and support to stand and walk is required (stage 1). Thereafter,

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there is a progressive motor and mental regression until the child reaches a final vegetative state (stage IV). Diagnosis of the disease and carrier detection are possible by means of ASA determination in leukocytes [7], urine [8], fibroblasts [9, 10], or serum [11]. Until now, no particular ethnic group has exhibited an increased frequency for any of the different types of MLD. In this study, we describe an increased frequency of late infantile MLD in a genetic isolate of Jews of Habbanite origin. This group emigrated to Israel from Habban (South Arabia, in the Southwest of the Hadhramaut) and was previously described by Bonné et al. and Bonné-Tamir and Ashbel [12–16]. The Jews of Habban have existed as a distinct endogamous tribe and religious community for many centuries. Almost the whole community, consisting of about 345 individuals, emigrated to Israel in 1950 and settled in a cooperative village. The community today retains its ethnic and social isolation. There are approximately 1,000–1,200 Habbanites presently in Israel; most are living in the cooperative village or have close family contact with it. The community is divided into four major patrilineages, two of which constitute almost 80% of the population (M. and H. lineages). The degree of consanguinity is very high (33% of first-cousin marriages). There is a tendency to marry not only inside the community but also within the lineage (77% of marriages within the M. lineage and 69% within the H. lineage).

In 1975, late infantile MLD was first diagnosed by us in a 3-year-old girl from this isolate. Furthermore, Bonné [12] described a Habbanite family in which five children died of a lethal neurodegenerative disease of an unknown origin. Those findings prompted us to screen the Habbanite community for affected children and carriers of late infantile MLD.

METHODS

Leukocytes were prepared according to the method of Kampine et al. [17] as previously described [18] from heparinized venous blood that had been collected in the village and transferred to our laboratory within 1 hr.

ASA was assayed according to Baum et al. [19], using β-nitrocatechol sulfate (Koch Light, Colnbrook, England) as a substrate. One unit of enzyme activity is defined as the amount of enzyme needed to hydrolyze 1 nmol of substrate/hr under conditions specified. In the first stage, seven parents of MLD-affected children were tested to establish the range of enzyme activity in obligate heterozygotes. The normal ASA activity was determined by assaying 33 adult non-Habbanite Jews living in the village. Average normal activity was defined as 186 U/mg protein ± 49 (SD), range = 95–292. The controls were in our laboratory's normal range from previous analysis. Average heterozygote activity was 63 U/mg protein ± 28 (SD), range = 31–116; the range comparable with previous determinations of obligate heterozygotes in our laboratory. MLD homozygotes had less than 10% of normal activity. Individuals were classified as inconclusive when ASA activity was between 89 (mean normal − 2 SD) and 119 U/mg protein (mean heterozygote value + 2 SD).

However, to minimize the inconclusive group, we used established information for further classification and the assumption that a new mutation is improbable. Thus, one of the parents of a defined heterozygote must also be heterozygous if the other parent is normal, and siblings of normal parents are also considered to be normal. Naturally, these conditions are based on the assumption that all the children in the community are legitimate, which was demonstrated by genetic analysis (Bonné-Tamir, personal communication, 1979).

RESULTS

Medical information from the years 1950 to 1975 indicated that eight children of this community died (seven in Israel and one in Habban) of a degenerative disease of the central nervous system clinically similar to MLD, although not biochemically diagnosed. Another four clinically typical cases of late infantile MLD were enzymatically diagnosed by us in 1975–1978. From 1950 to 1978, according to records and interviews with Habbanite families, there were 750–850 live births in the Habbanite community; thus, the approximate incidence of MLD in this community is at least 1/75 (1.3%) live births. The 12 MLD cases originated from six families, all of them from the two major patrilineages, H. and M. The genealogy of these families is shown in figure 1. Although a common ancestor is probable, it was not proven by our investigations.

As a result of our findings, a program for the screening of ASA levels in the adult Habbanite community was initiated. The results of screening approximately 65% of the adult population are summarized in table 1. Several individuals were assayed more than once, on different days, which resulted in only slight changes in their ASA values. However, six individuals who were previously categorized as inconclusive were

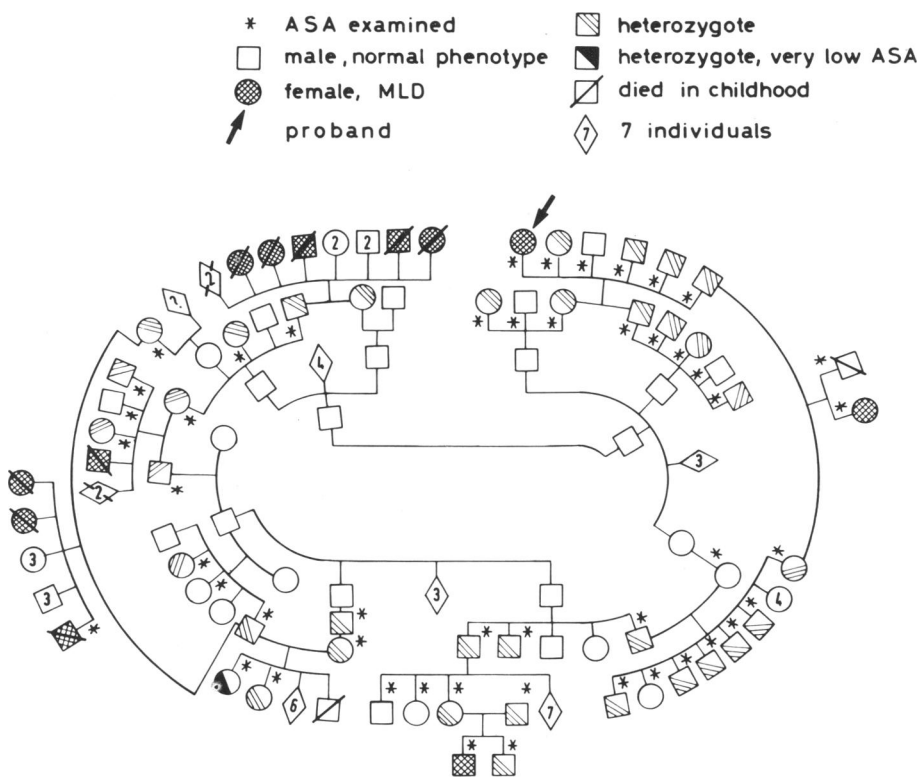

FIG. 1.—Pedigree of M. and H. families. Pedigree has been intentionally simplified throughout for clarity, and most members of these two families do not appear here although most were screened.

TABLE 1

SCREENING OF ASA LEVELS IN LEUKOCYTES IN THE JEWISH HABBANITE POPULATION

GENOTYPE	NO. INDIVIDUALS	%	ASA ACTIVITY*	
			Mean (± SD)	Range
Normal	204	78	191 ± 48	93–343
Heterozygote	46	17	69 ± 18	< 20–116
Inconclusive	13	5	...	94–117
Total	263	100	...	...

* U/mg protein.

unambiguously classified as normal upon a second determination. Thirty-six out of 46 heterozygotes were from the M. and H. lineages, and all were linked to the two common ancestors (fig. 1). We could not demonstrate a common link in the remaining heterozygotes. Also encountered in this survey was a case of a healthy adult with ASA values indistinguishable from the affected children; both parents and one sister of this individual were found with ASA values in the usual heterozygote range; the other sibs were not available for investigation.

From the adult population checked in the survey, 126 were married couples of whom both partners were Habbanites. The results of their screening are summarized in table 2. Among the nine couples at risk, two had children who died of a neurodegenerative disease, clinically similar to MLD, although not confirmed enzymatically. Another couple were the parents of the first case of MLD we diagnosed enzymatically, and six couples were identified heterozygotes by the screening.

The mother was of childbearing age in six out of the nine couples at risk. From 1976–1979, there were five pregnancies in four couples from this group. The first two cases, because of religious objections, refused amniocentesis, and both pregnancies resulted in the birth of MLD-affected children. In the other cases, amniocentesis was performed and the fetuses were diagnosed as unaffected.

DISCUSSION

Late infantile MLD is an autosomal recessive disease which has been reported in almost all ethnic groups [1]. Its incidence in Sweden was estimated to be 1/40,000 [20]. In 1970, four patients with MLD in a large kindred from an isolated community

TABLE 2

SCREENING OF 126 HABBANITE MARRIED COUPLES

Genetic status of couple	No. couples	%
Both partners homozygous normal	82	65.0
One partner heterozygote, one normal	28	22.2
One partner inconclusive, one normal	7	5.5
Both partners heterozygotes	7	5.5
One partner heterozygote, one inconclusive	2	1.6

in southwest Virginia were reported [21], but no increased frequency of the disease in any race or population is known. The incidence of 1/75 live births of late infantile MLD among Habbanite Jews, as reported here, is unusually high for a lethal recessive disease, and, thus, the Habbanite community constitutes a high-risk population for this disorder.

Tay-Sachs is the first and best-known example of prospective prevention of a genetic disease by mass screening for heterozygotes. Similarly, the program presented in this study for the detection of MLD carriers fulfills several preliminary conditions for such screening [22]: (1) the disorder occurs with increased frequency in a certain population group; (2) there is a relatively simple and accurate test for carrier detection; and (3) the disease can be diagnosed in utero early enough to terminate the pregnancy of an affected fetus. Our screening program of the Habbanite adult population found a carrier frequency for MLD of 1/6 (17%). The biochemical findings correlated completely with the genetic construction of the different families, so there were no discrepancies between the biochemical data and the data from the genetic pedigree. Six heterozygote couples with no affected children were identified by the screening, and amniocentesis was performed in three instances.

One individual with a very low ASA activity was identified in our screening, and several additional cases have been reported [23–27]. This emphasizes the need for routine examination of the enzyme profile in both parents of pregnancies at risk, since in this case, a pseudo-deficient fetus might be diagnosed as affected by the usual technique. In such cases, loading cultured cells with radioactive sulfatide and determination of the hydrolysis of the sulfatide as described by Kihara et al. [27] should be performed. The genetic implications of pseudo-deficiency of ASA as a result of our study are under evaluation.

There are several other similar populations in Israel in which certain genetic disorders are found in high frequency. The present study exhibits the possibility of a screening program for heterozygote detection to prevent the birth of affected homozygotes in such communities, depending, of course, on the technical possibilities to detect the disease and its carriers by a reliable unambiguous technique.

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REFERENCES

1. DULANEY JT, MOSER HW: Sulfatide lipidosis: metachromatic leukodystrophy, in *The Metabolic Basis of Inherited Disease*, 4th ed, edited by STANBURY JB, WYNGAARDEN JB, FREDRICKSON DS, New York, McGraw-Hill, 1978, pp 770–809
2. AUSTIN JH, BALASUBRAMANIAN AS, PATTABIRAMAN TN, SARASWATH S, BASU DK, BACKAWATT BK: A controlled study of enzymic activities in three human disorders of glycolipid metabolism. *J Neurochem* 10:805–816, 1963
3. MEHL E, JATZKEWITZ H: Cerebroside 3-sulfate as a physiological substrate of aryl sulfatase A. *Biochim Biophys Acta* 151:619–627, 1968
4. AUSTIN J, ARMSTRONG D, SHEARER L: Metachromatic form of diffuse cerebral sclerosis. The nature and significance of low sulfatase activity: a controlled study of brain, liver and kidney in four patients with metachromatic leukodystrophy (MLD). *Arch Neurol* 13:593–

614, 1965
5. WOLFE HJ, PIETRA GG: The visceral lesions of metachromatic leukodystrophy. *Am J Pathol* 44:921–930, 1964
6. BRAIN RW, GREENFIELD JG: Late infantile metachromatic leukoencephalopathy with primary degeneration of the interfascicular oligodendroglia. *Brain* 73:291–317, 1950
7. PERCY AL, BRADY RO: Metachromatic leukodystrophy: diagnosis with samples of venous blood. *Science* 161:594–595, 1968
8. AUSTIN J, MCAFEE D, SHEARER L: Metachromatic form of diffuse cerebral sclerosis. IV. Low sulfatase activity in the urine of nine living patients with metachromatic leukodystrophy. *Arch Neurol* 12:447–455, 1965
9. PORTER MT, FLUHARTY AL, KIHARA H: Metachromatic leukodystrophy. Aryl sulfatase A deficiency in skin fibroblast cultures. *Proc Natl Acad Sci USA* 62:887–891, 1969
10. KABACK MM, HOWELL RR: Infantile metachromatic leukodystrophy: heterozygote detection in skin fibroblasts and possible applications to intrauterine diagnosis. *N Engl J Med* 282:1336–1340, 1970
11. BERATIS BG, ARON AM, HIRSCHHORN K: Metachromatic leukodystrophy: detection in serum. *J Pediatr* 83:824–827, 1973
12. BONNÉ B: The Habbanite isolate —a study in human population genetics. *J Isr Med Assoc* (in Hebrew) 77:557–559, 1969
13. BONNÉ B: Merits and difficulties in studies of Middle Eastern isolates. *Isr J Med Sci* 9:1291–1298, 1973
14. BONNÉ B, ASHBEL S, MODAI M, ET AL.: The Habbanite isolate. I. Genetic markers in the blood. *Hum Hered* 20:609–622, 1970
15. BONNÉ B, ASHBEL S, BERLIN G, SELA B: The Habbanite isolate. III. Anthropometrics. Taste sensitivity and color vision. *Hum Hered* 22:430–444, 1972
16. BONNÉ-TAMIR B, ASHBEL S: The Habbanite isolate: demography and its genetic implications. *Man* (N. S.) 13:72–87, 1978
17. KAMPINE JP, BRADY RO, KANFER JN, FELD M, SHAPIRO D: Diagnosis of Gaucher's disease and Neimann-Pick disease with small samples of venous blood. *Science* 155:86–88, 1967
18. BACH G, KOHN G, LASCH EE, ET AL.: A new variant of mannosidosis with increased residual enzymatic activity and mild clinical manifestations. *Pediatr Res* 12:1010–1015, 1978
19. BAUM H, DODGSON KS, SPENCER B: The assay of aryl sulfatase A and B in human urine. *Clin Chim Acta* 4:453–455, 1959
20. GUSTAVSON KH, HAGBERG B: The incidence and genetics of metachromatic leukodystrophy in northern Sweden. *Acta Paediatr Scand* 60:585–590, 1971
21. BASS NH, WITMER EJ, DREIFUSS FE: Pedigree study of metachromatic leukodystrophy. *Neurology (Minneap)* 20:52–62, 1970
22. KABACK MM, NATHAN TJ, GREENWALD S: Tay-Sachs disease: heterozygote screening and prenatal diagnosis —U.S. experience and world perspective, *in Tay-Sachs Disease: Screening and Prevention*, edited by KABACK MM, New York, Alan R. Liss, 1977, pp 13–36
23. BUTTERWORTH J, BROADHEAD DM, KEAY AJ: Low aryl sulfatase A activity in a family without metachromatic leukodystrophy. *Clin Genet* 14:213–218, 1978
24. DUBOIS G, HARZER K, BAUMANN N: Very low sulfatase A and cerebroside sulfatase activities in leukocytes of healthy members of metachromatic leukodystrophy family. *Am J Hum Genet* 29:191–194, 1977
25. FLUHARTY AL, STEVENS RL, KIHARA H: Cerebroside sulfate hydrolysis by fibroblasts from a metachromatic leukodystrophy parent with deficient aryl sulfatase A. *J Pediatr* 92:782–784, 1978
26. LOTT IT, DULANEY JT, MILUNSKI A, HOEFNAGEL D, MOSER HW: Apparent biochemical homozygosity in two obligatory heterozygotes for metachromatic leukodystrophy. *J Pediatr* 89:438–440, 1976

27. KIHARA H, HO C-K, FLUHARTY AL, TSAY KK, HARTLAGE DL: Prenatal diagnosis of metachromatic leukodystrophy in a family with arylsulfatase A deficiency by the cerebroside sulfate loading test. *Pediatr Res* 14:224–227, 1980